IJBST

ISSN: 0974 – 3987
IJBST (2014), 7(3):15-24

The study on oogenetic stages in the ovary and fecundity during the phases of reproductive cycle of a female catfish, *Mystus cavasius* from Chambal River (Rajghat) Morena, Madhya Pradesh

Jaya Chaturvedi and D.N. Saksena
Aquatic Biology, Laboratory, School of studies in Zoology, Jiwaji University, Gwalior (M.P)
jaya2chaturvedi@gmail.com, dnsaksena@gmail.com

ABSTRACT
The study of oogenetic stages of catfish, *M. cavasius* has been done from September, 2011 to August, 2012. *M. cavasius* is a sexually dimorphic fish. Five oogenetic stages have been recognised in this fish species. They are Oocyte stage I (Chromatin-nucleolus stage), Oocyte stage II (Perinucleolus stage), Oocyte stage III (Yolk vesicle stage), Oocyte stage IV (Vitellogenic stage) and Oocyte stage V (Mature stage). The reproductive cycle of *M. cavasius* has been divided into five phases of histological changes. They include post- spawning phase (October- November), preparatory phase (December- February), maturing phase (March- April), Pre-spawning phase (May-June) and spawning phase (July- September). The study on fecundity has been done from May to August, 2012. The fecundity of female species was varied from 6442.68±1293.38 to 18,707.95±1355.59 depending on gonad weight, body weight and age of fish. The maximum fecundity was observed in month of July and lowest was observed in the month of August. The average fecundity per cm of body length was 871.17± 170.64 ova/ cm and average fecundity per gm of body weight was 231.28 ± 44.05 ova/gm.
Key words: Oogenetic stages, fecundity, *Mystus cavasius* and Chambal River.

INTRODUCTION

Reproduction is a very complex process that involves synchronized gametogenesis, development of the accessory reproductive organs and secondary sexual characters, migration to breeding grounds, courtship behavior, breeding etc. In most teleosts, the patterns of reproductive events are cyclic in nature. The maturation and spawning in fishes is adjusted to the most propitious time of the year ensuring maximum survival and faster growth of the young ones. Gonads are classified as ripe (mature) when eggs or milt were released when slight pressure was applied to the abdomen. During maturation an oogonium increases in size, due to the accumulation of ooplasm, and several cytological changes also takes place. A developing egg is called an oocyte, its several stages can be seen in a section of ovary. It is generally stated that oocyte development occurs in two phases: 1. Growth phase, which involves the increase in size of oocyte with some nuclear changes and 2. Vitellogenic phase, in which yolk is added to the oocyte. Yolk is a stored of nutritive material and is responsible for the growth of oocytes, differs widely from species to species in proportion and total amount of its proteins, fats and carbohydrate content during different phases of reproductive cycle. During oogenesis the oocyte accumulates reserve substances, which may be synthesized *in-ovo* denovo or may be transferred from the maternal circulation. Fecundity of any animal is an adaptation which ensures the survival of species under the condition in which it has been evolved, originated and lived. It also appears to bear some broad relationship to the care accorded to the eggs. Knowledge about fecundity of a fish has been an essential feature for evaluating the commercial potentialities of its stock, egg production, life history, culture and actual management of the fishery. The measure of fecundity in fishes is a basic determinant of productivity and contributes to the development of pisciculture. [1] stated that fecundity, among egg laying animals, is the number of eggs being studied for next spawning by a female. Fecundity of fish is variously related to the egg sizes, gonad size, length, weight of female fish and the age. It is assumed that large sized fish would be more fecund than the small sized fish. Recent work on the reproductive cycle and fecundity of female fish have been made by several workers in different fish species, *Paracheirodon axelrodi* [2], *Puntius filamentosus* [3], *Arius argyropleuron* [4], *Tilapia* species [5], *Terapon puta* [6], and *Amblypharyngodon mola* [7]. This fish is mainly consumed by the common people of the area due to its good nutritive value and moderate size. The population of this species is decreasing in its natural habitat due to continuous fish catching. Hence the Population of this fish species may be increase by the means of artificial breeding, so that it is important to know about the reproductive cycle of this species which is not studied so far. Therefore this study has been taken up to establish the histological changes in the ovary and importance of fecundity in reproductive cycle of a catfish, *M. cavasius.*

MATERIALS AND METHODS:

The female specimens of the catfish, M. cavasius were collected on monthly basis for a year overlapping two months i.e., from September, 2011 to August, 2012 near Rajghat, Chambal River by using a cast net taking the help of local fishermen. The specimen were wiped with a towel to remove the water and mucous. Thereafter, the length and weight of the specimen were taken. For the study of female reproductive organs, reproductive cycle of the female fish both the morphological and histological studies were done for histological studies of the ovaries six fishes were sacrificed every month throughout a period of one year. The weight of ovaries was taken with the help of spatula balance in gm. The anterior, middle and

posterior portion of ovaries were taken for their fixation purposes in the different fixatives used were aqueous Bouin's fluid, Hollande's modified fluid and alcoholic Bouin's fluid for a prescribed time period. After fixation, the ovarian tissue was washed thoroughly under running tap water until the colour of the fixative was removed and the ovaries were preserved in 70% cellosolve for further processing. The ovaries were dehydrated with the ascending grade of cellosolve i.e., 80%, 90% and 100% cellosolve. Two changes for 30 minutes were given in each grade of cellosolve in the series. After dehydration, the material was cleared in methyl benzoate and benzene for 30 minutes. The tissue was then transferred to a solution of wax dissolved in benzene. The impregnation was done in molten wax (melting point 60-62^0C) for at least for 3 hours (3 changes of 1 hour duration each). The wax blocks were prepared after the impregnation was over. The wax blocks containing ovarian tissue were kept in a mixture of glycerine and water (1:1) overnight after trimming and exposing the tissue before sectioning. The sections were cut at 5-6µm thickness. The sections were stained by Delafield's haematoxylin and eosin stain and then examined under the light microscope. For the analysis of the ovarian cycle, the oogenetic stages of oocytes development were recognized *viz.*, chromatin nucleolus stage, perinucleolus stage, yolk vesicle stage, vitellogenic stage and mature stage, following the observations of [8] and with the help of these oogenetic stages the reproductive cycle of a fish species can be estimated. Histologically, the ovarian cycle was determined on the basis of histological picture of the ovary in different months and stages of different oogenetic stages in connective tissue etc. The fecundity of fish was estimated by using a formula:

Fecundity = Average number of ova in the subsamples of ovary x Weight of ovary in gm.

RESULTS:

The catfish, *Mystus cavasius,* is a sexually dimorphic fish. The female specimens of this species are bright in colour and having a bulging abdomen. The ovaries are enclosed in a thin peritoneal covering which is outermost layer of ovary the peritoneal layer is followed by tunica albuginea composed of collagenous and smooth connective tissue fibres interspersed with smooth muscle fibres and the blood vessels. The inner most layer of ovary is known as germinal epithelium consists of single layer of cells disposed into ovigerous lamellae containing various oogenetic stages. In all five stages have been described in *M. cavasius* based on the observations made by [8].

Oogenetic stages

a. **Chromatin nucleolus stage:** This stage is characterised by presence of large, spherical central nucleus occupying most of the cell. The chromatin material is present in the form of thin thread like structure in the nucleus. A single nucleolus is located at the periphery of the nucleus. A very thin layer of cytoplasm is present which is basophilic in nature. The chromatin nuclear stage is present in the form of nests or groups. A thin follicular layer may also be present surrounding the oocyte (shown in fig. 1).
b. **Peri-nucleolus stage:** The oocytes in this stage are surrounded by a flattened follicular layer. Nucleus is large and spherical in shape. Nucleus is increased in size and nucleoli are increased in number. Chromatin material becomes granular and basophilic, and dispersed around. Nucleoli arranged peripherally on the inner surface of the nucleus. Cytoplasm is slightly basophilic (fig.2).
c. **Yolk vesicle stage:** This stage of oocyte development is featured by the appearance of vacuolated zone in the ooplasm along the periphery. These vacuoles are known as yolk vesicles. These vesicles appear first in the nuclear region and then middle part of the cytoplasm. The nuclear membrane appears in the form of irregular outline and nucleoli are present in its crenulations. Some nucleoli have seen extruded into the ooplasm but are not far from the nucleus. The follicular layer becomes more thickened and a very thin vitelline membrane appears surrounding the ooplasm (fig. 3).
d. **Vitellogenic stage:** In this stage the cytoplasm of the oocyte lost its basophilic nature and the nuclear membrane has completely disappeared. The yolk vesicles and yolk granules appear in the whole cytoplasm. The yolk globules are appeared in a ring in the cytoplasm. The yolk globules become acidophilic in nature as oocyte grow in size. The yolk protein gets accumulated in the ooplasm which is responsible for the growth of whole oocyte. The yolk globules are seen to be deposited on the outer margins of the oocyte. The vitelline membrane is more prominent. The theca layer also appears around the follicular layer. The nuclear layer may become irregular in shape (fig. 4).
e. **Mature stage:** This stage is characterised by the large amount of yolk in the cytoplasm forming a uniform layer in which the nucleus is not clearly visible. The oocyte is bounded by three layer outermost layer is called theca layer, middle one is called follicular layer or zona granulosa while innermost layer is called vitelline membrane or zona radiata. The nucleus is migrated towards the periphery

from the centre for mitotic division. The nuclear membrane is completely disappeared (fig.5).

The ovary, besides these five stages also have two more stages *viz*., atretic follicles and post-ovulatory or discharged follicles.

(a) Atretic follicles: The immature oocytes which are not able to reach maturity and the mature oocytes which are not able to spawn and undergo for atresia are called atretic follicles. The atretic follicles are characterised by shrinkage of oocytes due to liquification of yolk, having disintegrated vitelline membrane and absorption of yolk globules. Hypertrophied follicle layer and theca layers are observed (fig. 6).

(b) Post-ovulatory follicles: The post-ovulatory follicles or ruptured follicles are formed as a result of the extrusion of the mature oocytes from the ovary. Empty follicles were seen in the ovary. The residual eggs were undergone for atresia. The yolk content is completely disappeared and whole content of oocytes is absorbed in the ovary leaving behind hypertrophied theca and granulosa layer (fig. 7).

Histological changes in the ovaries during the reproductive cycle:

1. **The post –spawning phase (October-November):** This stage running from the month October to November. During this phase of reproductive cycle the ovigerous lamellae are seen vary much distinct which contain oocyte in stage I and oocyte in stage II predominantly. The blood vascular supply is decreased and connective tissue is increased in the ovaries. Some of the oocytes are seen undergoing for atretic degeneration (fig. 8).
2. **The preparatory phase (December-February):** The ovary shows lamellae having nests of oogonia and oocytes in stage I, II and III can be seen. The atretic follicles have also been evident in this stage (fig. 9).
3. **The Maturing phase (March-April):** The blood capillaries become conspicuous and increased vascular supply. The connective tissue is quite evident in February, little in March and least in April. Oocytes in Stage I and II are lesser in number as compared to preparatory phase and oocyte in stage III and IV are commonly present. The atretic follicles are also observed in this phase (fig. 10).
4. **The pre-spawning phase (May-June):** The ovaries during this phase consist mainly of yolky oocytes in stage III, IV and V. The blood supply is increased considerably but the ovigerous lamellae are not appeared. Oocytes in stage IV and V are observed in abundance. The nucleus of the oocytes starts moving towards the periphery for meiotic division or germinal vesicle break down (fig 11).
5. **The spawning phase (July-September):** The oocytes in stage IV, V and post-ovulatory follicles are present in this phase of reproductive cycle. The ovarian wall is thin but is more vascularised. Most of the ovaries shown post-ovulatory follicle phase of the reproductive cycle during the September. Some atretic follicular stage is also observed in mature ovaries (fig. 12).

Fecundity:

The fecundity of female species was varying from 6442.68±1293.38 to 18707.95±1355.59 ova/ fish depending on the weight of gonad and maturity stages. The highest fecundity of *M. cavasius* was observed in the month of July (18707.95±1355.59 ova/ fish) and lowest in the month of August (6442.68±1293.38 ova/ fish) (table 3 and fig. 4). The average fecundity per cm of body length was 871.17± 170.64 ova/ cm and average fecundity per gm of body weight was 231.28 ± 44.05 ova/gm (table 1 and fig. 13). The fecundity increases with the increasing in the gonad weight. It is highly positively correlated with the gonad weight.

DISCUSSION:

The catfish, *M. cavasius* is a sexually dimorphic fish and female specimens of this species having bulging abdomen. The sexual dimorphism is greatly pronounced in the breeding season in *M. cavasius*. [9] has described the eleven stages of oocyte development in *Liopsetta obscura viz.*, chromatin nucleous stage, early perinucleolus stage, late perinucleolous stage, yolk vesicle stage, primary yolk stage, secondary yolk stage, migratory nucleus stage, pre-maturation stage, maturation stage and ripe stage. [10] in *Glossogobius giurus*, [3] in *Puntius filamentosus* and [11] in *Xenentodon Cancila* have described ten stages in oogenesis following the scheme of [9]. Nine stages of oogenesis have been recognized by [12] in *Gudusia chapra*. [2]in *Paracheirodon axelrodi* have showed the eight oogenetic stages. [13] in *Limanda ferruginea* and [14] have recognized seven stages of oocyte development. In *Empetrichthys latos* [15] six stages of oogenesis were described. Five stages of oogenesis have been recognised in *Notopterus chitala* [16], and *Amblypharyngodon mola* [7]. In present study on *M. cavasius* five oocyte developmental stages *viz.*, oocyte stage I (chromatin nucleolus stage), oocyte stage II (perinucleolus stage), oocyte stage III (yolk vesicle stage), oocyte stage IV (vitellogenic stage), oocyte stage V (mature stage) and atretic follicles and post-

ovulatory follicles during oogenesis showing similarity with the recent findings of [8] and [15].

Teleosts exhibit seasonal changes in the gonads leading to the production of mature gametes. Teleosts show a cyclical pattern of reproduction and in general they almost all are annual breeders. Several workers have described the annual reproductive cycle on the basis of different phases in the reproductive cycle. In *Epinephelus diacanthus* [17] and *Xenentodon Cancila* [11] three phases of reproductive cycle were observed. [2] and [4] have distuingished the four phases in the reproductive cycle. Five phases of reproductive cycle were recorded in *Leptobotia elongate* [18] and *Tilapia* species [5]. A female catfish, *Mystus cavasius* show five phases in the reproductive cycle *viz.*, post spawning phase (October- November), Preparatory phase (December-February), Maturing phase (March-April), pre-spawning phase (May-June) and spawning phase (July-September) have been distinguished on the basis of histological and morphological observations of ovary.

The fecundity of a fish has shown a positive relationship with the weight of ovary and body weight. The maximum number of eggs was observed in the month of July when the ovary reaches its peak maturity stage. The prediction is that spawning takes place in the month of July and highest spawning is in the month of August, when average number of matured ova was observed minimum. The average fecundity estimated was 13936.44±2768.927 ova/fish and the mean fecundity per cm of body length has been 871.17± 170.64 ova/cm and mean fecundity per gm of body weight has been 231.28 ± 44.05 ova/gm. It exhibited a strong correlation with the gonad weight. The fecundity of *Hilsha ilisha* from Bay of Bengal is significantly correlated with the body length; body weight and gonad weight [19]. The fecundity of *Epinephelus diacanthus* showed that the gonad weight and the total fecundity had a significant linear relationship [17]. In *Garra rufa*, [20] have observed a significant relationship between fecundity and fish size and also between absolute fecundity and gonad weight. [21] have reported that the fecundity of *Labeo calbasu* was increasing with the increase in the fish length, fish weight and gonad weight. The similar observations were also made by [22] on the fecundity of this fish and found that the mean fecundity was 12432.38 ± 3401.92. In our study the mean fecundity was 13936.44±2768.927 ova/fish of this species which shows similarity with the mean fecundity (12432.38 ova/fish) obtained by [22] on the same fish species.

CONCLUSIONS:

The whole study about the reproductive organs, reproductive cycle of *M. cavasius* revealed that *M. cavasius* is sexually diamorphic fish, usually breeds during rainy season from July to September. The oogenesis undergoes under five oogenetic stages *i.e.*, chromatin nucleolus stage, perinucleolus stage, yolk vesicle stage, vitellogenic stage, mature stage and atretic follicle stage and postovulatory follicle stage were also present. There are five phases in the reproductive cycle of *M. cavasius* were observed *i.e.*, post-spawning phase, preparatory phase, maturing phase, pre-spawning phase and spawning phase. As regard to the fecundity of the fish which was maximum during July while minimum in August.

ACKNOWLEDGEMENTS

We are thankful to the Head of the department of Zoology, Jiwaji University, Gwalior for providing all the essential laboratory facilities. I am also thankful to the University for awarding “University Research Fellowship” for financial support during the course of my study.

REFERENCES:

[1] Royce, W F. 1972. Introduction to the Fishery Science. Academic Press, New York, 251p.

[2] Brito, MFG., and Bazzoli, N. (2009). Oogenesis of the cardinal tetra *Paracheirodon axelrodi* (1956) a histological and histochemical study. Braz J Morphol Sci, 26**(1):** 14-18.

[3] Mannan, M. Mannar, Maridass, M., and Thangarani, S. (2010). Gonad developmental cycle of *Puntius filamentosus.* Int J Biolo Technol, 1**(2):**69-77.

[4] Isa Mansor Mat, Noor Nurul Shafikah Mohd, Yahya Khairun, and Nor Siti Azizah Md. (2012). Reproductive biology of estuarine catfish, *Arius argyropleuron* (Siluriformes: Ariidae) in the northern part of Peninsular Malaysia. J Biol Agricul Healthcare, 2**(3):** 14-27.

[5] El-Kasheif Midhat, Shalloof Kariman A.Sh. and Authman Mohammad M.N. (2013). Studies on some reproductive characters of *Tilapia* species in Damietta branch of the river Nile, Egypt. J Fish and Aquat Sci.

[6] Nandikeswari, R., and Anandan, V. (2013). Analysis on gonadosomatic index and fecundity of Terapon puta from Nallavadu coast Pondicherry. Int J Scient Res Publications, 3, 1-4.

[7] Gupta S. and Banerjee S. (2013). Studies on some aspects of Reproductive biology of *Amblypharyngodon mola* (Hamilton-Buchanan, 1822**).** Int Res J Biolo Sci, 2**(2):** 69-77.

[8] Mahmoud, Hatem H. (2009). Gonadal Maturation and Histological Observations of *Epinephelus areolatus* and *Lethrinus nebulosus* in Halaieb/Shalatien Area “Red Sea”, Egypt.

[9] Yamamoto K. (1956). Studies on the formation of the fish eggs. I. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura*. J Fac Sci Hokkaido Univ Ser VI Zool, (**12):** 362-376.

[10] Saksena, D.N. (1976). Reproductive organs and reproductive cycle of female Indian freshwater goby, *Glossogobius giuris* (Ham.). Zool Jb Ant, **(96):** 45-73.

[11] Bano, Z., Manohar S., Chauhan, R., Bhat Najeeb, A., and Qureshi, T. A. (2012). Annual Changes in the

Ovary of *Xenentodon Cancila* (Ham.). Int J Envi Sci, 2**(3):** 1239-1245**.**

[12] Rahman, M.A. and Haque, M.M. (2008). Gonadal development of *Gadusia chapra* (Hamilton) from Rajdhala reservoir. Bangladesh. J Inland Fish Soc Ind, 40**(1):** 10-15.

[13] Howell, W.H. (1983). Seasonal changes in the ovaries of adult yellow tail flounder, *Limanda ferruginea.* Fish Bull, 81**(2):** 341-355.

[14] Sehriban, C., Bromage, N., Randall, C., Rana, K. (2001). Oogenesis, Hepatosomatic and Gonadosomatic Indexes, and Sex Ratio in Rosy Barb (*Puntius conchonius*). Turk J Fisher Aqua Sci, **(1):** 33-41.

[15] Uribe Mari Carmen, Grier Harry J., and Parenti Lynne R. (2012). Ovarian Structure and oogenesis of the oviparous goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (Teleostei, Cyprinodontiformes*).* J Morphol, **(273):**371–387.

[16] Kohinoor, A.H.M., Jahan, D.A., Khan, M.M., Islam, M.S. and Hussain, M.G. (2012). Reproductive biology of black feather chital, *Notopterus chitala* cultured in a pond of Bangladesh. Int J Agril Res Innov Tech, 2 **(1):** 26-31.

[17] Rao, C., and Krishnan, L. (2009). Studies on the reproductive biology of the female spiny cheek grouper, *Epinephelus diacanthus* (Valenciennes, 1828). Indian J Fish, 56**(2):** 87-94.

[18] Xia Yin Zing, Racey Paul, Li Jian, and Guang Zhang Yao. (2012). The Ovarian Cycle of the Fish *Leptobotia elongata* Bleeker, Endemic to China. Pakistan J Zool, 44**(4):**997-1005.

[19] Saifullah, A. S. M., Rahman S. Md., Khan, Y. and Ahmed, S. 2004. Fecundity of *Hilsa ilisha* (Ham.) from the Bay of Bengal. Pakistan J. Biol. Sci., 7**(8):** 1394-1398.

[20] Abedi Masoud, Houshang Shiva Amir and Malekpour Rokhsareh (2011). Reproductive biology and age determination of *Garra rufa* Heckel, 1843 (Actinopterygii: Cyprinidae) in central Iran. Turk J Zool,, 35**(3):** 317-323.

[21] Mishra, Shailja and Saksena, D.N. 2012. Gonadosomatic index and fecundity of an Indian major carp, *Labeo calbasu* in gohad reservoir. The Bioscan, Int J Quat Life Sci, 7**(1):** 43-46.

[22] Protap Kumar, Roy and Hossain, M.A. 2006. The fecundity and sex- ratio of M*ystus cavasius* (Hamilton) (Cypriniformes: Bagridae). J Life Earth Sci, 1**(2):** 65-66.

Table 1: The mean contribution of fecundity, fecundity/ cm of body length and fecundity/ gm of body weight of *M. cavasius* from May 2012- August 2012

Months	Fecundity	Fecundity/ cm of bodylength	Fecundity/ gm of bodyweight
May	13132.55±1388.06	811.28±78.34	217.17±22.20
June	17462.56±1136.55	1097.32±59.27	287.75±18.10
July	18707.95±1355.59	1162.80±83.20	307.581±19.73
August	6442.68±1293.38	413.28±84.30	112.63±23.79

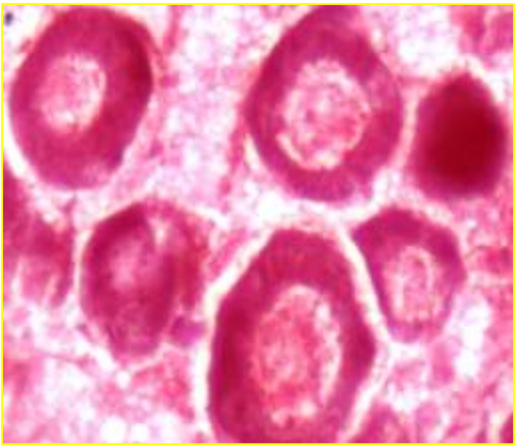

Fig. 1 Transverse section of ovary showing the oocyte stage I (chromatin nucleolus) stage of oogenesis

ISSN: 0974 – 3987
IJBST (2014), 7(3):15-24

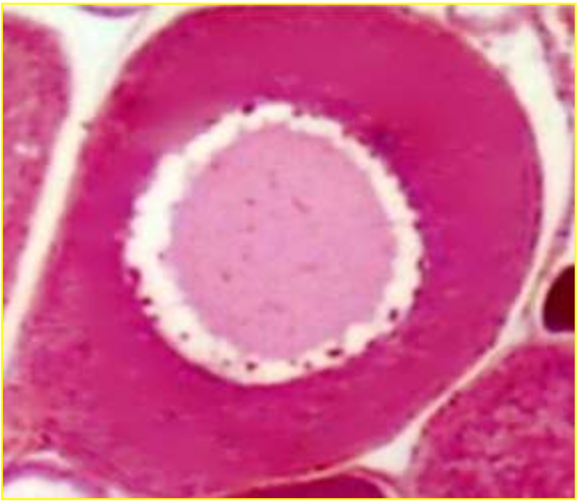

Fig. 2 Transverse section of ovary showing the oocyte stage II (peri-nucleolus stage) of oogenesis

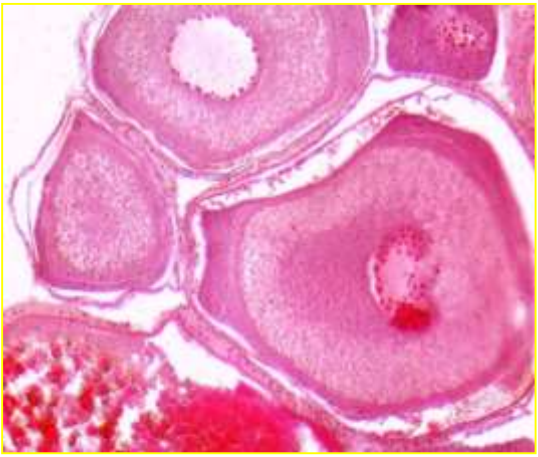

Fig. 3 Transverse section of ovary showing the oocyte stage III (Yolk vesicle stage) of oogenesis

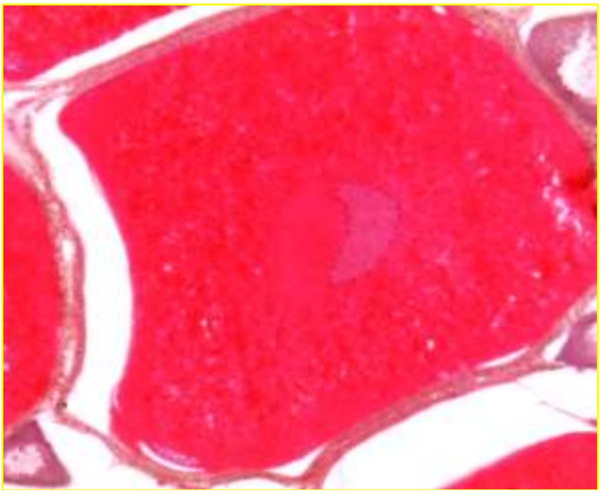

Fig. 4 Tranverse section of ovary showing the oocyte IV (vitellogenic stage)

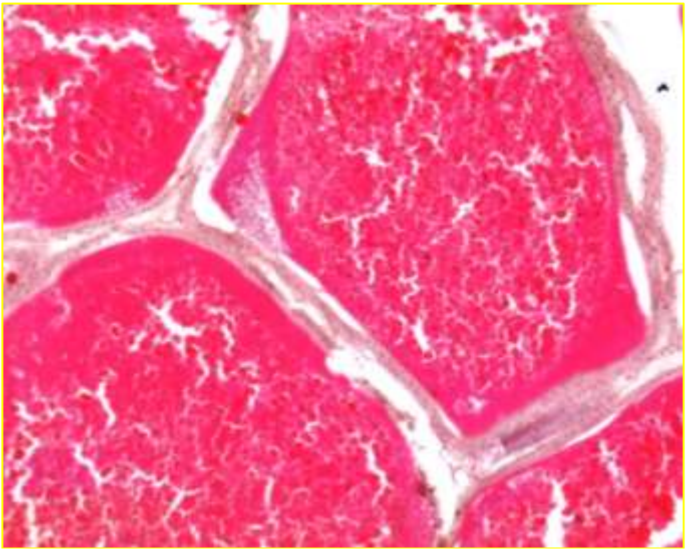

Fig. 5 Transverse section of ovary showing an oocyte stage V(Mature stage) of oogenesis

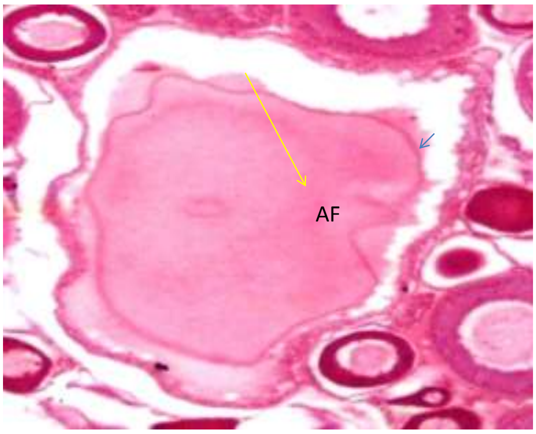

Fig. 6 Transverse section of ovary showing atretic follicular stage of oogenesis, AF = atretic follicles

Fig. 7 Transverse section of ovary showing post-ovulatoryfollicles stage of oogenesis, PF = post-ovulatory follicles

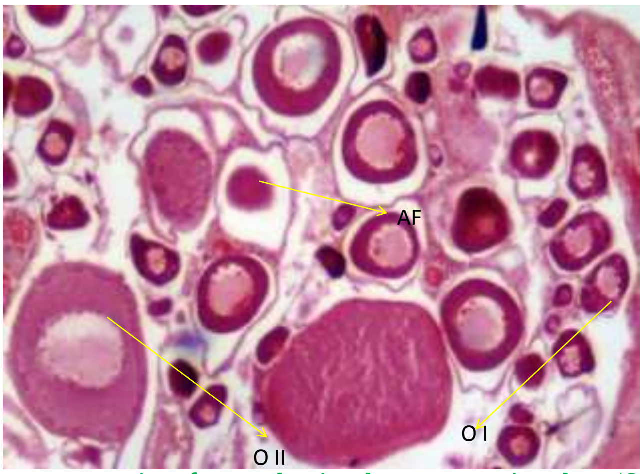

Fig. 8 Transverse section of ovary showing the post-spawning phase (October-November) of the reproductive cycle OI = oocyte stage I, OII = oocyte stage

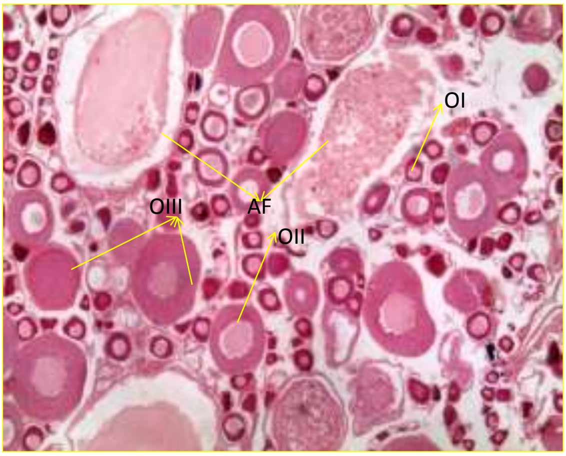

Fig. 9 Transverse section of ovary showing the preparatory phase (December-February) of the reproductive cycle, OI = oocyte stage I, OII = oocyte stage II, OIII = oocyte stage III

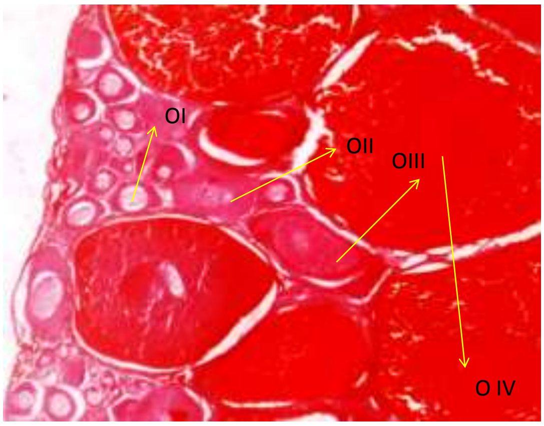

Fig. 10 Transverse section of ovary showing the maturing phase (March-April) of the reproductive cycle, OI = oocyte stage I, OII = oocyte stage II, oocyte stage IV = oocyte stage IV

ISSN: 0974 – 3987
IJBST (2014), 7(3):15-24

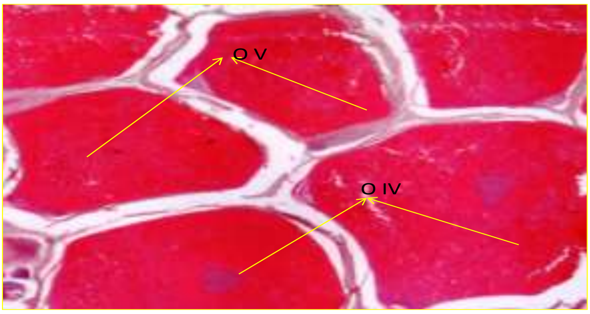

Fig. 11 Transverse section of ovary showing the pre-spawning phase (May-June) of the reproductive cycle, O IV = oocyte stage IV, OV = oocyte stage V

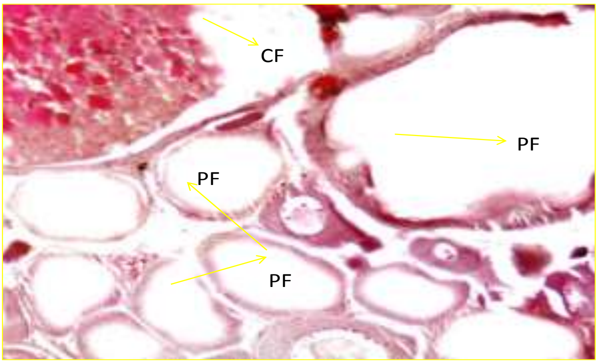

Fig. 12 Transverse section of ovary showing spawning phase of reproductive cycle, PF = post-ovulatory follicles, CF = caves of post-ovulatory follicles

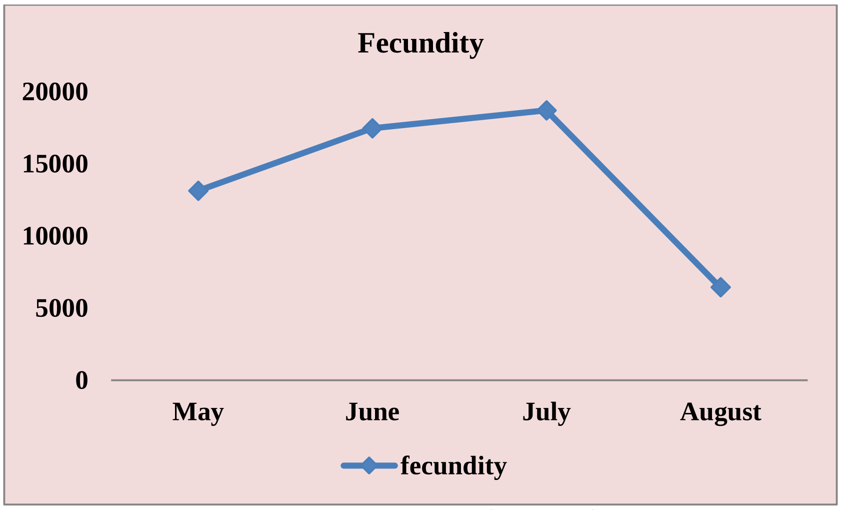

Fig. 13: The seasonal variations in the fecundity of *M. cavasius*